Functional Analysis of the TCR$\alpha^-\beta^+$ Cells That Accumulate in the Pneumonic Lung of Influenza Virus-Infected TCR-$\alpha^{-/-}$ Mice[1]

Maryna Eichelberger,* Anthony McMickle,* Marcia Blackman,* Peter Mombaerts,[2†] Susumu Tonegawa,† and Peter C. Doherty[3]*

*Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38101; and †Howard Hughes Medical Institute at the Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

In mice homozygous (−/−) for a targeted TCR-α gene disruption, some thymocytes express a cell-surface TCR-β chain on the cell surface in the absence of a TCR-α chain, and a few CD4$^+$CD8$^-$ TCR-$\alpha^-\beta^+$ cells accumulate in the peripheral lymphoid organs. We have infected these mutant mice with an influenza A virus to show that large numbers of TCR-β^+ cells (most of which are CD4$^+$) can be retrieved from the pneumonic lung. Both freshly isolated TCR-$\alpha^-\beta^+$ cells and TCR-$\alpha^-\beta^+$ hybridoma cell lines derived from influenza virus-infected mutant mice respond appropriately to stimulation with anti-CD3ϵ or the Mls-1 superantigen. It thus seems that CD4$^+$ TCR-$\alpha^-\beta^+$ cells in the peripheral lymphoid organs of TCR-α mutant mice can signal through their TCR surface complex. However, there are no indications that CD4$^+$ TCR-$\alpha^-\beta^+$ lymphocytes can either recognize a complex between MHC and influenza virus peptide or act as effector or Th cells. The existence and function of such cells in wild-type mice remains to be established. *The Journal of Immunology*, 1995, 154: 1569–1576.

Mice homozygous (−/−) for mutant TCR-α or -β genes (1, 2) offer obvious possibilities for analyzing the functional characteristics of $\gamma\delta$ T cells operating in the absence of $\alpha\beta$ T cells. These mice were originally used to demonstrate the role of the α and β TCR chains in T cell development: in TCR-$\alpha^{-/-}$[4] mice thymocytes proceed to the CD4$^+$8$^+$ stage, whereas T cell development does not continue substantially beyond the

CD4$^-$8$^-$ stage in the absence of an intact β chain (1, 2). The role of the TCR-β chain in promoting thymocyte development was further demonstrated by the generation of TCR-β transgenic RAG-1 or RAG-2 mutant mice (1, 3), supporting the finding that TCR-β gene expression precedes that of TCR-α during T cell development (4, 5).

Surface expression of the β gene product in the absence of an α-chain has been described for immature thymocytes from normal fetal mice (6), for thymocytes from TCR-β transgenic SCID (7), RAG-1 (1), or RAG-2 (3) mutant mice, as well as for transformed CD4$^+$8$^+$ thymocyte lines (8, 9). Initial studies of $\alpha^-\beta^+$ T cells, using iodination of cell surface proteins followed by immunoprecipitation with a β-specific Ab, indicated that the β chain was expressed on the cell surface as a homodimer (8). However, it has recently been demonstrated that the β chain is disulfide-linked to a protein that cannot be iodinated, but which can be detected as a 33-kDa glycoprotein when biotinylated and immunoprecipated from a SCID thymocyte line transfected with a rearranged β gene (10). In TCR-$\alpha^{-/-}$ mice, CD3ϵ^+4$^+$8$^-$ dull TCR-β^+ cells, which depend on class II MHC for their development, can be detected in all peripheral lymphoid organs (1, 11). No function has been ascribed yet to these CD4$^+$8$^-$ TCR-$\alpha^-\beta^+$ lymphocytes, nor have they been detected in wild-type mice.

The lymphocyte component of the inflammatory exudate obtained by bronchoalveolar lavage (BAL) of normal C57BL/6J (B6) mice infected intranasally (i.n.) with the HKx31 (H3N2) influenza A virus is dominated by CD8$^+$ and CD4$^+$ T cells expressing TCR-$\alpha\beta$ heterodimers (12–14) with much smaller numbers of CD4$^-$8$^-$ $\gamma\delta$ and $\alpha\beta$ T cells also present (15, 16). Either the virus-specific CD4$^+$ or CD8$^+$ $\alpha\beta$ T lymphocytes can clear the infection after depletion of the other T cell subset (12, 13, 17). Evidence of viral specificity and/or in vivo effector function has yet to be demonstrated for the CD4$^-$8$^-$ $\alpha\beta$ or $\gamma\delta$ lymphocytes. We have addressed the question of a possible biologic function of $\gamma\delta$ T cells acting in the absence of the TCR-$\alpha\beta^+$ set by infecting the TCR-$\alpha^{-/-}$ mice with the HKx31 influenza A virus. During the course of these experiments, we observed that, in addition to $\gamma\delta$ T cells, large numbers of CD4$^+$ TCR-$\alpha^-\beta^+$ cells accumulate in the pneumonic lung, providing an opportunity to analyze the responsiveness of this unusual population in vitro and in vivo.

Materials and Methods

Mice

The TCR-$\alpha^{-/-}$ mice were developed at the Massachusetts Institute of Technology (1). Homozygotes and heterozygotes were sent to St. Jude Children's Research Hospital to establish a breeding colony. These mice are derived genotypically from two (H-2^b) strains, 129/Sv and B6. Both male and female TCR-$\alpha^{-/-}$ mice were infected with the HKx31 influenza virus at 10 to 30 wk of age, and the TCR-$\alpha^{+/-}$ littermates were used as controls. Apart from the infection with influenza virus, all mice were maintained under specific pathogen-free conditions.

Virus infection and titration

The A/HKx31 (H3N2) influenza A virus (18) was grown in the allantoic cavity of chick embryos and shown to be free of bacteria, mycoplasma, and endotoxin. Mice were infected i.n. under Avertin (2,2,2-tribromo-ethanol) anesthesia with 30 μl of PBS containing 240 viral hemagglutinating units, which causes a severe (but generally nonlethal) infection in normal B6 or 129/Sv mice (12, 17). Titration of virus from homogenized mouse lungs was done using embryonated chicken eggs; the titer was determined as the EID$_{50}$, i.e., the dilution at which 50% of the inoculated eggs contained virus (12). The titer of virus-specific Ab in serum samples was determined by hemagglutination inhibition (19).

Sampling the mice

Pooled BAL cells were obtained from the lungs of virus-infected mice, as described previously (12). The mediastinal lymph nodes (MLN) were then removed and whole lungs were frozen for virus titration. The MLN and BAL cell suspensions were washed and counted. The BAL population was also adhered on plastic for 90 min at 37°C to remove macrophages, with further analysis concentrating on the nonadherent population composed principally of lymphocytes.

Lymphocyte phenotyping

The lymphocytes were stained for flow cytometry (12) and then analyzed using LYSIS on a FACScan (Becton Dickinson, Mountain View, CA) in two-color mode. The mAbs used for staining were 2C11 anti-CD3ϵ (20), H57.597 anti-TCR-β (21), GL3 anti-TCR-δ (22), 53.4.6 anti-CD8 (23), RM-4-5 anti-CD4 (24), MEL-14 anti-L-selectin (25), 2D7 anti-CD11a (26), 16A anti-CD45RB (27), H1.2F3 anti-CD69 (28), and RA3.6B2 anti-B220 (29). The H57.597 and GL3 mAbs were either biotinylated, in which case streptavidin-Red613 (Life Technologies Inc., Gaithersburg,

MD) was used as a second-step reagent, or conjugated directly to phycoerythrin. The other mAbs were all conjugated to FITC. These reagents were purchased from PharMingen (San Diego, CA).

T cell hybridomas

Lymphocytes were recovered from the BAL and MLN (12) of infected TCR-$\alpha^{-/-}$ mice. The cells were then stimulated for 3 days with Con A (2 μg/ml) in supplemented S-MEM (Life Technologies Inc.) containing 10% FCS and supernatant from PMA-stimulated EL4 cells and fused using 50% polyethylene glycol (Boehringer Mannheim, Indianapolis, IN) with the TCR-$\alpha^-\beta^-$ BW5147 cell line (30). The hybrids were selected in complete S-MEM containing hypoxanthine-aminopterin-thymidine. Cultures derived from single colonies were analyzed with FACScan. Surface TCR-β expression was determined using H57.597 (21), and the Vβ used was analyzed with a panel of Abs (14) specific for Vβ 2, 3, 5.1/5.2, 6, 7, 8.1/8.2/8.3, 8.1/8.2, 8.2, 9, 11, 13, or 14. Intracellular immunofluoresence with a hamster pan-anti-TCR-α reagent, 710.16 (kindly provided by Dr. R. Kubo, Cytel Inc., La Jolla, CA), was performed to confirm the absence of TCR-α in the hybridoma cells.

The hybridoma cell lines were tested for specificity by exposure of 2 $\times$ 10^4 cells to 1 $\times$ 10^5 AM11 (H-2^b) transformed macrophages (14) which were uninfected, pulsed with purified protein derivative (PPD; 50 μg/ml), or infected for 3 h with the HKx31 influenza A virus. Stimulation with irradiated YCD3 hybridoma cells (31), which produce a mAb to CD3ϵ, was used to measure the response through the TCR. After 24 h, supernatants were assayed for IL-2 production by measuring ^{3}H-thymidine uptake in CTL line cells. A separate set of experiments analyzed their capacity to respond to superantigens Mls-1 (expressed on CBA/J but not CBA/Ca spleen cells) and staphylococcal enterotoxin B (SEB; Sigma Chemical Co., St. Louis, MO). IL-2 release was measured in culture supernatants using reduction of MTT as a measure of proliferation of HT2 cells, as previously described (32). In this assay, one U of human rIL-2 is equivalent to 160 U. The class II MHC$^+$ CH12 B cell lymphoma (H-2^a, I-A^k, I-E^k) was used as the presenting cell for the SEB (10 μg/ml).

T cell cultures

Cells recovered from the MLN of infected TCR-$\alpha^{-/-}$ mice were depleted of B cells using anti-mouse Ig rat Ab conjugated to Dynabeads. D1-LP (H-2^b) splenocytes were treated with LPS (30 μg/ml) and rIL-4 (25 U/ml) overnight, and mitomycin C-treated before using as a source of Mls-1 stimulators (32). Stimulators were added to lymphocyte suspensions in 24-well plates using 5 $\times$ 10^5 responders with 5 $\times$ 10^6 stimulators in 2.5 ml in the presence or absence of 10 U/ml rIL-2. The surface phenotype and Vβ usage of freshly isolated T cells and lymphocytes cultured for 5 days were determined by immunofluorescence.

Results

CD4$^+$ TCR-β^+ cells accumulate in the lungs of influenza-infected TCR-$\alpha^{-/-}$ mice

Control TCR-$\alpha^{+/-}$ mice show the pattern of predominant CD8$^+$ and CD4$^+$ $\alpha\beta$ T lymphocyte extravasation (Fig. 1, *left-hand panels*) into the bronchoalveolar space that is characteristic of normal B6 mice infected with the HKx31 influenza A virus (12). At this late time point (day 22 postinfection), the virus had been cleared and the inflammatory cell population diminished to approximately 1.7 $\times$ 10^5 cells/mouse lung. The number of cells lavaged from each $\alpha^{-/-}$ mouse lung (from which virus was not cleared) was approximately 10 times higher (1.75 $\times$ 10^6 cells/mouse). These BAL populations contain substantial numbers of CD4$^+$8$^-\beta^+$ T cells (25% of lymphocyte population) as well as $\gamma\delta$ T cells (60%) which were either CD4$^-$8$^+$ or CD4$^-$8$^-$ (Fig. 1, *right panels*). Although CD4$^+$8$^-\beta^+$ T cells are present in the BAL population from uninfected TCR-$\alpha^{-/-}$ mice of the same age, the total
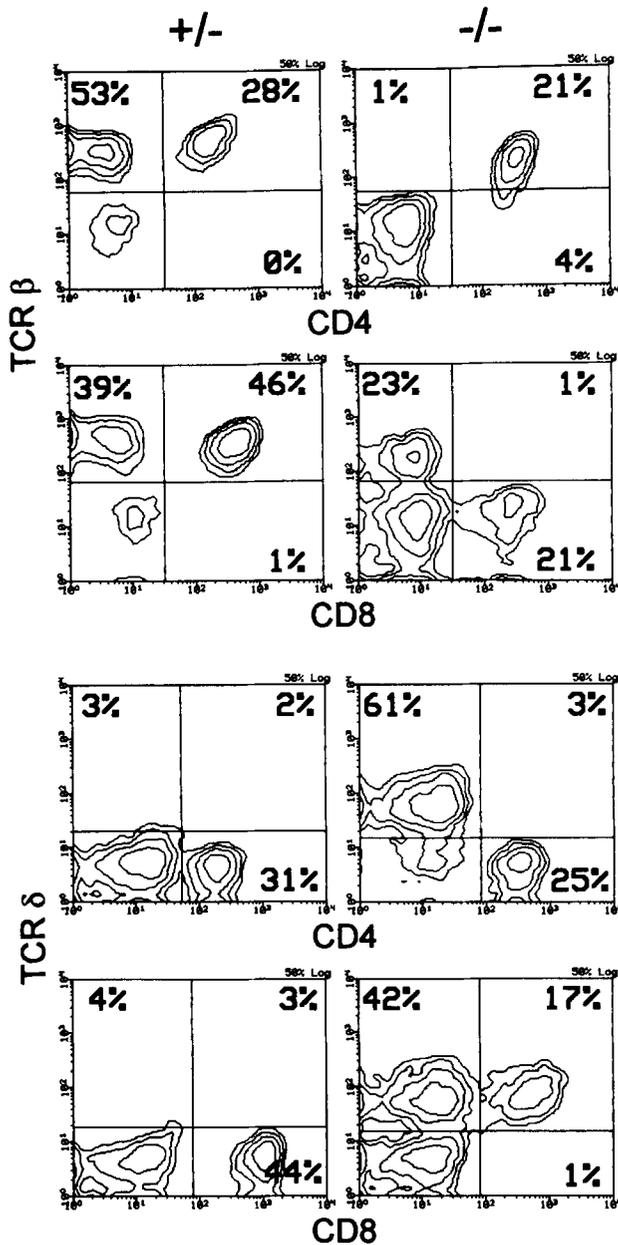
FIGURE 1. The CD4, CD8, TCR-β, and TCR-δ phenotypes are shown for nonadherent BAL cells recovered from TCR-$\alpha^{+/-}$ control and TCR-$\alpha^{-/-}$ mice sampled 22 days after i.n. infection with the HKx31 influenza A virus.

FIGURE 2. Two-color flow cytometric analysis is shown for BAL lymphocytes stained with mAbs to TCR-β, CD3ϵ, and TCR-δ. The mice were infected 21 days previously.

number of cells that can be lavaged from each lung is very small (1 to 10 × 10⁴ cells/mouse). The Ab used for immunofluorescence, H57.597, did not stain any cells in the BAL of influenza-infected mice that had a mutant TCR-β chain (data not shown). As with influenza-infected control mice, very few B cells are detected in the BAL of TCR-$\alpha^{-/-}$ mice. Pooled BAL from TCR-$\alpha^{-/-}$ mice contained >20% TCR-β^+ lymphocytes and >30% $\gamma\delta$ T cells (Fig. 1 and data not shown from three separate experiments).

Both the TCR-β^+ and TCR-$\gamma\delta^+$ lymphocytes were also present in the draining TCR-$\alpha^{-/-}$ MLN. Although the to-

tal number of cells/MLN in TCR-$\alpha^{-/-}$ mice was comparable with the values for $\alpha^{+/-}$ mice, the regional nodes of the mutant mice contained relatively few T lymphocytes. In four separate experiments, the TCR-β^+ population in the MLN 2 to 3 wk after infection was 4 ± 3% in $\alpha^{-/-}$ mice compared with 54 ± 7% in controls, whereas the TCR-δ^+ set was 9 ± 5% in $\alpha^{-/-}$ compared with 1.5 ± 0.5% in controls. The predominant population in the MLN of virus-infected TCR-$\alpha^{-/-}$ mice was B220$^+$ B cells. Thus, neither the TCR-β^+ nor the $\gamma\delta$ T lymphocytes expanded in numbers to compensate fully for the absence from the lymph node of the $\alpha\beta$ T cell subsets, although both populations are recruited to the virus-infected lung. Flow cytometric analysis established that the TCR-β^+ lymphocytes in the TCR-$\alpha^{-/-}$ mice were uniformly positive for expression of CD3ϵ, suggesting the presence of a functional TCR (Fig. 2, upper panels). A possible explanation for the presence of TCR-β^+ lymphocytes in the TCR-$\alpha^{-/-}$ mice is that the TCR-β chain had paired with TCR-δ (3). This was not supported by flow cytometric analysis, which showed that the cells staining with the H57.597 mAb to TCR-β and the GL3 mAb to TCR-δ are distinct populations (Fig. 2, lower panels). A population of CD3$^+$ cells which does not stain for TCR-β or TCR-δ was also detected and will be investigated further.

Freshly isolated TCR-β^+ lymphocytes recovered from the BAL of the TCR-$\alpha^{-/-}$ mice at 22 days after infection tended to be somewhat larger and stained less brightly for TCR-β but more strongly for CD4 than the inflammatory CD4$^+$ $\alpha\beta$ T cells from the TCR$^{+/-}$ controls (Fig. 3). The predominance of CD4 expression in the TCR-β^+ cell population is not absolute: mice depleted of CD4$^+$ cells with

MLN   BAL

Forward Light Scatter

TCR β Fluorescence Intensity

CD4 Fluorescence Intensity
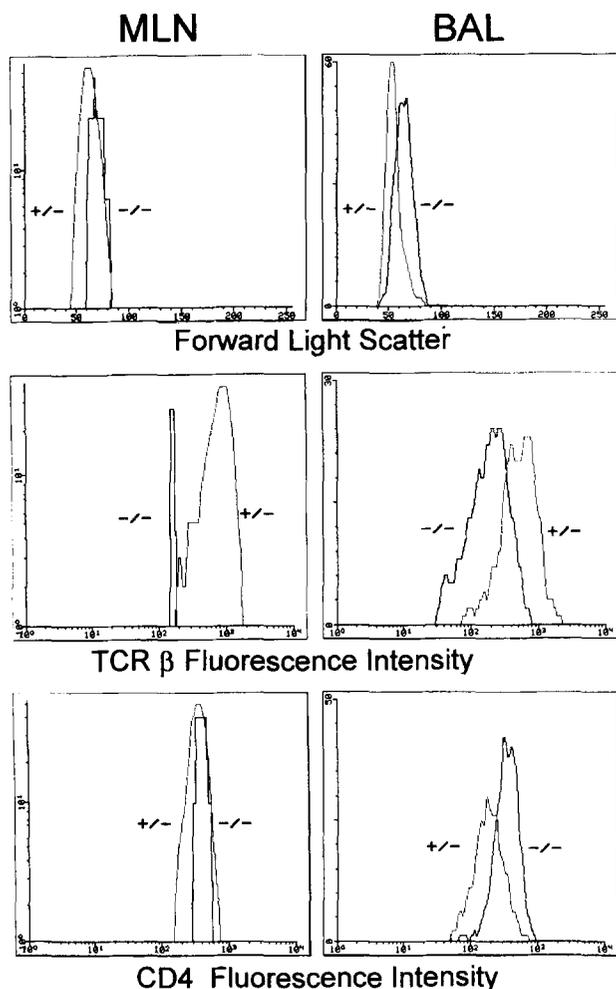
FIGURE 3. The TCR-β^+ lymphocytes from the MLN and BAL of TCR-$\alpha^{+/-}$ and $^{-/-}$ mice infected i.n. 22 days previously were compared for size (forward light scatter) and intensity of staining for TCR-β and CD4.

the GK1.5 mAb (12) and subsequently infected with A/HKx31 showed different degrees of depletion of the β^+ population 20 days after infection. All of the remaining β^+ T cells in the MLN were CD4$^-$8$^-$. In addition, a population of CD4$^-$8$^+\beta^+$ T cells was present in the MLN of TCR-$\alpha^{-/-}$ mice that had been treated with mAb GL3 to deplete $\gamma\delta$ T cells (results not shown).

The TCR-β^+ cells in the BAL were CD62L(L-selectin)$^-$, CD45RB$^-$ (Fig. 4), the characteristic phenotype of activated T cells that accumulate at the inflammatory site during viral infections (33). Like the $\alpha\beta$ T cells from BAL of TCR-$\alpha^{+/-}$ mice, the $\alpha^-\beta^+$ cells had increased expression of CD11a and CD69 (Fig. 4), both characteristics of activated cells. In a separate experiment, only a small population of these cells expressed TSA, an Ag expressed on cortical thymocytes (data not shown), indicating that they are mature. This is not surprising, as their development is dependent on class II MHC molecules (11), indicating that

they have experienced some selective pressure and are not immature cells that have "leaked" into the periphery.

A panel of hybridomas was then made from the pooled BAL population recovered from five influenza-infected TCR-$\alpha^{-/-}$ mice. We derived 28 hybridoma cell lines, 21 of which were TCR-β^+, 5 others expressed a TCR-δ chain, and the remainder appeared to have no TCR. The TCR-β^+ hybridomas phenotyped by flow cytometry were Vβ6$^+$(7 of 16), Vβ8.3(2/17), Vβ8.2(1/17), and Vβ14(1/17). In five instances, the V regions could not be identified with the available monoclonal reagents. None of the hybridomas expressed more than one Vβ specificity, indicating that the TCR is not a heterodimer of two different β chains.

TCR-β^+ cells respond to superantigens but not to viral Ags

All of the hybridomas produced IL-2 when stimulated via CD3ϵ (Table I). However, we have not been able to demonstrate any Ag specificity. None of the clones responded to syngeneic macrophages that were infected with the HKx31 virus used to infect the mice, which was still present in the respiratory tract at the time the BAL was recovered. Because hsp65 mRNA is up-regulated in the infected lung (34), we asked whether syngeneic macrophages pulsed with PPD would act as stimulators: there was no response (Table I). Some of the hybridomas showed a relatively high level of spontaneous IL-2 production, which remained consistent throughout a number of experiments (data not shown).

The TCR-β on the hybridoma cells is able to promote IL-2 production following exposure to an appropriate superantigen (Table II). In agreement with previously well-established Vβ/superantigen correlations (35), the cells expressing Vβ6 all responded to Mls-1, and two Vβ8 hybridomas responded to SEB. One Vβ6 hybridoma also responded to SEB. This rare reactivity has previously been reported (36). Unexpectedly, one Vβ8.3 hybridoma responded to Mls-1 but was not stimulated by SEB. The experiment was repeated with the same results.

Freshly isolated β^+ T cells from the lymph node also responded to Mls-1 as expected (Table III), with a Vβ6 population becoming predominant in both control and $\alpha^{-/-}$ cultures and some expansion of a Vβ8.1 population. However, the response to Mls-1 by $\alpha^-\beta^+$ cells was somewhat different in that the Vβ8.2 cells expanded to a greater extent than those of the control. The number of β^+CD8$^+$ cells in the TCR-$\alpha^{-/-}$ mice was very small, and in vitro culture did not result in expansion of a CD8$^+$ population. Although the number of cells recovered from Mls-1-stimulated TCR-$\alpha^{-/-}$ cultures was less than for TCR-$\alpha^{+/+}$ cultures, the number of $\alpha^-\beta^+$ cells in the freshly isolated $-/-$ population was small (Table III); therefore, the rate at which these two populations expanded was approximately equivalent. The proliferative response of BAL cells
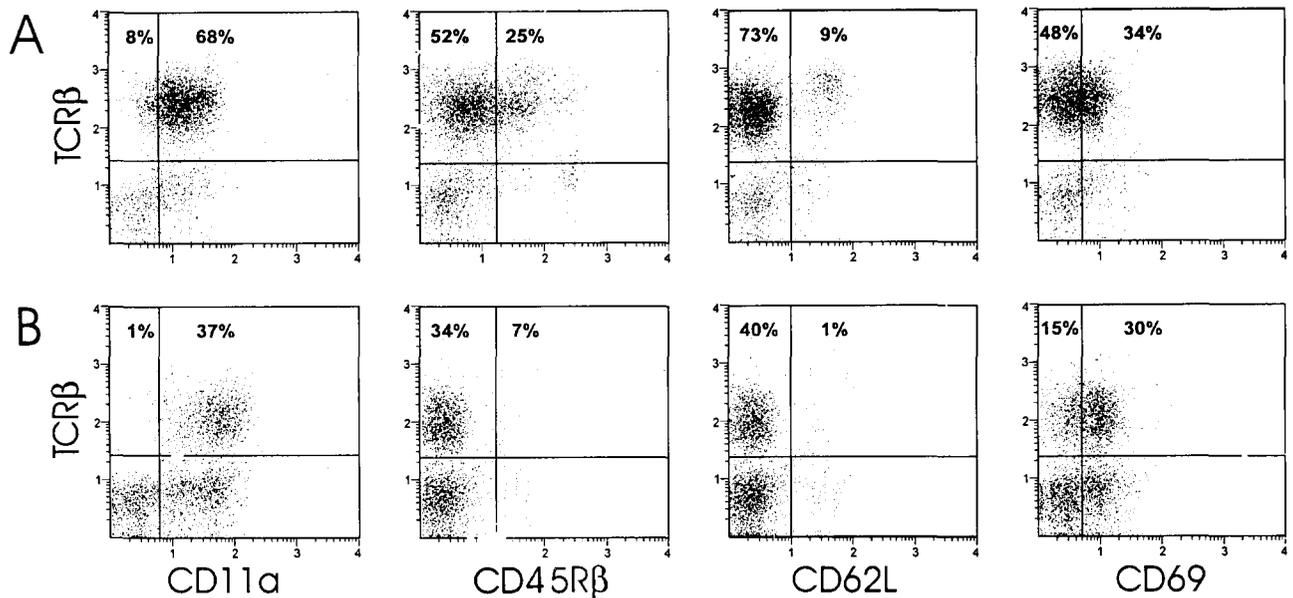
FIGURE 4. Two-color flow cytometric analysis is shown for BAL lymphocytes stained with mAbs to TCR-β (vertical axis) and CD11a, CD45RB, CD62L, and CD69 (horizontal axis). *A* shows cells pooled from TCR-$\alpha^{+/-}$ mice; *B* shows cells pooled from TCR-$\alpha^{-/-}$ mice that had been depleted of $\gamma\delta$ T cells by treatment with GL3. Both groups were infected with A/HKx31 20 days before sacrifice. The small shift with mAb to CD69 is a consequence of the dilution used in this experiment, repeat experiments with greater amounts of mAb gave better separations of CD69 negative and positive populations. The profiles were similar when cells from undepleted TCR-$\alpha^{-/-}$ mice were used in the analysis.

Table I. *IL-2 secretion by hybridoma cell lines derived from the BAL of HKx31-infected TCR-$\alpha^{-/-}$ mice in response to TCR cross-linking and conventional Ags*

Clone	Vβ TCR	Anti-CD3 (YCD3)	AM11 Cells[a] A/HK×31	AM11 Cells[a] PPD	AM11 Cells[a] Nil
1.8	6	171 ± 26	18.4 ± 3.5	14.2 ± 0.5	20.5 ± 4.3
1.22	6	148 ± 16	23.9 ± 0.9	20.9 ± 0.7	26.9 ± 3.7
1.26	6	158 ± 16	3.8 ± 2.1	2.9 ± 0.2	4.3 ± 1.7
1.29	6	167 ± 31	2.4 ± 0.1	2.7 ± 0.5	3.2 ± 0.7
1.36	6	168 ± 24	3.6 ± 0.7	2.3 ± 0.2	2.9 ± 0.6
1.40	6	147 ± 18	2.8 ± 0.3	2.2 ± 0.6	3.6 ± 0.5
1.6	8	179 ± 22	28.2 ± 22	24.4 ± 0.5	37.8 ± 1.8
1.31	8.2	185 ± 37	11.3 ± 0.6	13.2 ± 1.7	15.7 ± 2.1
1.2	8.3	164 ± 23	3.3 ± 0.6	3.0 ± 0.8	4.3 ± 2.9
1.19	ND[b]	145 ± 10	2.8 ± 0.1	2.7 ± 0.4	3.6 ± 2.2
1.55	ND	145 ± 9	25.9 ± 1.5	26.9 ± 1.6	29.9 ± 1.7

[a] The transformed H-2^b macrophage cell line AM11 (class I$^+$ II$^+$) was used to present influenza A virus (A/HKx31) or hsp65-containing PPD. AM11 cells in the absence of additional Ag (Nil) was used as a negative control. Cells were infected for 3 h with A/HKx31 before addition of hybridoma cells. Influenza virus-specific $\alpha\beta$ T cell hybridomas give an optimum response with this infection regimen. PPD was used at a final concentration of 50 μg/ml. IL-2 contained in the supernatants of triplicate cultures was measured using ^{3}H-thymidine uptake in CTL line cells. The results are expressed as mean ± SD × 10^2 cpm for ^{3}H-thymidine uptake.

[b] ND, not determined.

to Mls-1 was similar to that found for the lymph node. These experiments were repeated with comparable results.

Absence of detectable T cell helper function and virus recovery

The most obvious role of CD4$^+$ $\alpha\beta$ T cells in virus infections is to provide help for the Ag-specific B cell re-

Table II. *Response characteristics (U/ml of IL-2) of hybridoma cell lines to superantigens*

Clone	Vβ TCR	Normal Spleen CBA/J	Normal Spleen CBA/Ca	CH12.1.16 Cells +SEB	CH12.1.16 Cells −SEB	Anti-CD3[a]
1.80	6	1280	0	0	0	160
1.22	6	640	0	0	0	320
1.26	6	640	0	0	0	640
1.29	6	2560	0	40	0	1280
1.36	6	640	0	0	0	160
1.40	6	640	0	0	0	80
1.60	8.3	0	0	320	0	320
1.31	8.2	0	0	1280	0	160
1.2	8.3	320	0	0	0	320
1.19	ND[b]	0	10	0	0	160
1.55	ND	0	0	0	0	40

[a] Hybridomas were incubated with immobilized 2C11 mAb to CD3 (32).

[b] ND, not determined. The hybridomas were positive for H57.597 staining but negative for Vβ2, Vβ3, Vβ4, Vβ5.1, Vβ5.2, Vβ6, Vβ7, Vβ8.1, Vβ8.2, Vβ8.3, Vβ9, Vβ11, Vβ13, and Vβ14.

sponse (37). The virus-specific Ab titers in serum were determined by hemagglutination inhibition. After 22 days of infection, six of the control mice showed hemagglutination inhibition at 1:160 to 1:640 dilution of serum, whereas samples from four TCR-$\alpha^{-/-}$ mice tested on the same day were uniformly negative. There was thus no indication that the CD4$^+$ TCR-β^+ lymphocytes in the TCR-$\alpha^{-/-}$ mice can act as helpers. Weak agglutination at 1:10 dilution of serum was sometimes evident, and was correlated with a virus-specific IgM response (results not shown).

Table III. *Stimulation of freshly isolated MLN CD4⁺ T cells[a] with Mls-1*

Cell Source	In Vitro Stimulation	IL-2 (10 U/ml)	Cells/well (×10⁵)	% CD4	% CD4⁺ Cells Staining for Vβ			
					Vβ6	Vβ8.1	Vβ8.2	Vβ8.3
+/+	None[b]	+	5.0	17	7	7	17	5
	Mls-1		23.0	20	45	28	14	5
	Mls-1	+	40.0	22	44	33	11	1
−/−	None[a]	+	5.0	16	19	6	9	7
	Mls-1		3.2	47	54	13	34	0
	Mls-1	+	6.4	48	56	13	34	3

[a] After removal of cells using anti-mouse Ig Dynabeads, the +/+ MLN cell suspension contained 73% β⁺ T cells, 4% δ⁺ cells, and 10% B220⁺ cells. The −/− population contained 16% β⁺ T cells, 30% δ⁺ cells, and 44% B220⁺ cells. The cells were plated at 5 × 10⁵ cells/well.

[b] Cultures labeled "None" had normal, irradiated B6 splenocytes added as feeders and were supplemented with IL-2. A proliferation analysis using propidium iodide was done with the Mls-1 + IL-2 population: 45% of the +/+ and 47% of the −/− cells were in cycle.

Table IV. *Influenza virus is not cleared from lungs of TCR-α⁻/⁻ mice*

Expt.	Day Postinfection	Group	No. Mice	log₁₀EID₅₀
1	22	+/−	8	None
		−/−	5	3.9 ± 3.0
2	21	+/−	3	None
		−/−	3	5.0 ± 0.5
3	14	+/−	7	None
		−/−	5	4.8 ± 0.8

Normal mice that lack CD8⁺ (but not both CD8⁺ and CD4⁺) T cells can eliminate influenza virus from the respiratory tract (15). Virus recovery from the lungs of control and TCR-α⁻/⁻ mice is shown in Table IV. The TCR-α⁻/⁻ mice were unable to clear the infection. By these criteria, neither the CD4⁺ TCR-β⁺ nor the γδ T cells of the TCR-α⁻/⁻ mice act as effectors to clear virus from the lung.

Discussion

A population of dull TCR-β⁺, mostly CD4⁺, cells has been demonstrated to accumulate in the periphery of TCR-α⁻/⁻ mice (1). The numbers of these β⁺ lymphocytes increase with age (11). Perhaps, they proliferate and accumulate as a consequence of exposure to environmental Ags. This accumulation may not reflect conventional Ag specificity of the cells, but rather an ability to proliferate at sites of virus-induced inflammatory pathology.

Analysis of the thymus from the TCR-α⁻/⁻ mice has shown that the spectrum of TCR-β gene rearrangement is as extensive as in the TCR-α⁺/⁻ controls (1). However, only a fraction of TCR-α⁻/⁻ thymocytes stains faintly with the H57.597 mAb to TCR-β. The few CD4⁺ TCR-β⁺ cells that are found in peripheral lymphoid tissue maintain a pattern of low surface TCR expression, although this is higher than for cells in the thymus. Compared with cells isolated from normal mice, staining for TCR-β is also relatively weak in the substantial population of TCR-β⁺ lymphocytes that accumulates in the lungs of TCR-α⁻/⁻ mice with influenza pneumonia. Furthermore, the inflammatory TCR-α⁻β⁺ cells tend to be larger and stain more brightly for CD4 than normal αβ lymphocytes.

To examine the existence of these TCR-α⁻β⁺ cells in wild-type mice, a mAb is required that allows detection of surface TCR-α chains. Although it is unlikely that this would comprise a significant population, it is of interest to ask whether cells that have only a β component of the TCR can function in any way. An in vivo infectious process is a useful way to test this, as a range of functional readouts is available. The CD4⁺ TCR-β⁺ lymphocytes in TCR-α⁻/⁻ mice indeed show many of the physiologic characteristics of conventional T lymphocytes. The TCR complex is clearly able to transduce an inductive signal following protein-binding to the TCR-β chain, as both freshly isolated cells and hybridomas respond appropriately to the Mls-1 superantigen. However, superantigen stimulation has been shown to activate signal transduction pathways that are biochemically distinct from those elicited by stimulation with conventional Ags (38–40).

Like normal T cells, the CD4⁺ TCR-α⁻β⁺ set is able to localize to a site of inflammatory pathology. This is a substantial phenomenon, as the CD4⁺ TCR-α⁻β⁺ lymphocytes constitute 20 to 40% of the T cells in the BAL of TCR-α⁻/⁻ mice with influenza pneumonia. However, the fact that these lymphocytes accumulate in the pneumonic lung does not in any sense establish that the process is mediated by an Ag-specific TCR. Most T cell localization to sites of virus-induced pathology is secondary to damage (or cytokine production) by a few virus-specific effectors, or a consequence of direct virus-induced changes in the endothelial and epithelial barriers which gate lymphocyte extravasation from the blood (41). For example, the BAL of influenza-infected conventional mice depleted concurrently of the CD4⁺ and CD8⁺ subsets still contains significant (although greatly reduced) numbers of lymphocytes, at least some of which are producing cytokines (42).

No indications were found from either in vitro or in vivo analysis that the CD4⁺ TCR-α⁻β⁺ lymphocytes are responding specifically to Ag. The hybridoma cell lines were not stimulated by virus-infected stimulator cells: the same was true for lymphocytes recovered directly from the lung (data not shown). There was no evidence of Th cell activity for virus-specific B lymphocytes, and in vivo elimination of the CD4⁺ subset did not modify the spectrum of

virus recovery from infected lung. It seems that, at least as far as influenza virus is concerned, expression of a TCR-α chain is required to generate a functional receptor capable of binding a peptide–MHC glycoprotein complex. It is possible that the $\alpha^-\beta^+$ T cells play a more subtle role. We are currently investigating the possibility that they may limit the severity of the disease, perhaps via cytokine-mediated effector mechanisms.

Acknowledgments

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